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The biodiversity of *Aspergillus* section *Flavi* and aflatoxins in the Brazilian peanut production chain

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ABSTRACT

A total of 119 samples of peanut were collected throughout the peanut production chain in São Paulo State, Brazil. The peanut samples were directly plated for determination of percentages of infection and a polyphasic approach was used to identify *Aspergillus* section *Flavi* species. Further, the potential for aflatoxin production by the isolates was tested using the agar plug technique and the presence of aflatoxins in peanuts was assessed using an immunoaffinity column followed by quantification using HPLC with reverse phase column and fluorescence detection. The limit of detection and quantification were 0.05 and 0.17 μg/kg for total aflatoxins, respectively. Four species of *Aspergillus* section *Flavi* were isolated: *A. caelatus* (11), *A. flavus* (515), *A. parasiticus* (17) and *A. tamarii* (13). All isolates of *A. parasiticus* were able to produce aflatoxin B and G whereas aflatoxin B was produced by 50% of *A. flavus* isolates. Aflatoxins were found in 12 samples at concentrations ranging from 0.3 to 100 μg/kg. The data reported in this study add information on the occurrence and biodiversity of fungi in peanuts at several stages of the production chain. The occurrence of aflatoxins is also of major relevance for continuous monitoring and assessment of likely exposure of consumers to aflatoxins through consumption of peanuts.

1. Introduction

Brazil is the third largest peanut producer in the Americas, behind the United States and Argentina (USDA, 2015). The state of São Paulo stands out as the largest national producer and approximately 80% of the peanuts produced in the state are exported to European countries such as the Netherlands, United Kingdom and Russia, and Algeria in Africa (Martins, 2013; CONAB, 2015).

Aspergillus section *Flavi* occurrence in the peanut production chain represents a major concern due to the production of aflatoxin by some species such as *Aspergillus flavus* (aflatoxin B producer) and *Aspergillus parasiticus* (aflatoxin B and G producer). Produced by molds, aflatoxins are secondary metabolites which are the most potent carcinogens known according to the *International Agency for Research on Cancer* (IARC) as group 1 (IARC, 2002). As peanut contamination by aflatoxins is a well-known problem, some countries have established limits in peanuts for this toxin. The European Community commission established that there must be a maximum of 15 μg/kg total aflatoxins in peanuts for sorting, or other physical treatment, before human consumption or use as an ingredient in foodstuffs, and 4 μg/kg for peanuts processed and ready to eat (EC, 2006). The Brazilian Health Surveillance Agency (ANVISA) established limits of 20 μg/kg total aflatoxins in peanuts used for further processing and ready to eat peanuts (ANVISA, 2011).

Aflatoxigenic fungi and aflatoxin contamination in peanuts have been reported in several countries such as Brazil (Freitas & Brigido, 1998; Sabino et al., 1999; Nakai et al., 2008; Gonçalez et al., 2008), Argentina (Vaamonde, Patriarca, Pinto, Comerio, & Degrossi, 2003; Asis, Barrionuevo, Giorda, Nores, & Aldao, 2005; Pildain et al., 2008), United States of America (Xue et al., 2003; USDA, 2015), Uganda (Kaaya, Eigel, & Harris, 2006), Democratic Republic of Congo and South Africa (Kamika, Mngqawa, Rheeder, Teffo, & Katerere, 2014), Malawi (Matumba et al., 2014), Egypt (Youssef, El-Maghraby, & Ibhaim, 2008) and Pakistan (Mushtaq, Sultana, Anwar, Khan, & Ashrafuzzaman, 2012) among others. However, few studies have used the polyphasic taxonomic approaches which enable the distinction of new species in *A.* section *Flavi* described recently (Samson et al., 2014). Moreover, studies have agreed that prevention of aflatoxigenic fungi comprises the best method to reduce aflatoxin contamination in peanuts (Goldblatt, 1971; Dickens, 1977; Torres, Barros, Palacios, Chulze, & Battilani, 2014). To improve and ensure low aflatoxigenic fungal contamination and consequent aflatoxin production, knowledge of ideal conditions for the production and assessment of occurrence of these fungi at every stage of the production chain is important.

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Therefore, the aim of the present study was to isolate and identify species of *Aspergillus* section *Flavi* throughout the peanut production chain using a polyphasic approach (morphological and physiological characters, extrolite data and molecular analysis); to investigate the ability of aflatoxin production by the isolates and assess the presence of aflatoxins in peanut samples from the field to ready-to-eat.

2. Materials and methods

2.1. Peanut and soil samples

Peanut samples, each of approximately 2 kg, were collected at different stages of the production chain directly from farms and processing plants located in the state of São Paulo, Brazil during 2013 and 2014. A total of 119 samples were collected at: field stage (n = 27) which included uprooting (n = 3), windrow (n = 9), pulling (n = 11), and transport (n = 4), and from processing stage: drying (n = 19), threshing (n = 11), sorting (n = 25), blanching (n = 21), and ready-to-eat (n = 16). Six soil samples from peanut fields were also analyzed. The number of samples collected depended on the availability at each stage during the sampling days.

2.2. Water activity

The peanut and soil water activity was determined using an Aqualab Series 3TE instrument (Decagon, Pullman, WA, USA) at 25 ± 1 °C, in duplicate.

2.3. Fungal isolation

From each 2 kg of the collected sample, approximately 100 g were taken randomly. These sub-samples were disinfected superficially by immersion in 0.4% sodium hypochlorite solution for 2 min. Then fifty pieces of nuts were sampled randomly and plated onto Dichloran 18% Glycerol Agar (DG18), according to the methodology of Pitt and Hocking (2009). The plates were then incubated for 5 days at 25 °C. For soil samples the dilution plating method was used, according to Pitt and Hocking (2009). From each sample, 25 g were added under aseptic conditions to 225 mL of sterile peptone water 0.1%. Aliquots of the serial dilutions were inoculated onto plates containing DG18, following incubation at 25 °C for 7 days.

2.4. Morphological examination

After incubation, the plates were examined and all the fungal species were first isolated in Petri plates containing Czapek Yeast Autolysate (CYA) agar at 25 °C for 7 days to be later identified by specific protocols for each genus. *Aspergillus* section *Flavi* were examined on standard identification media for *Aspergillus* species (CYA), at 25 °C, 37 °C and 42 °C and on *Aspergillus flavus/parasiticus* agar (AFPA) at 25 °C for 7 days (Pitt & Hocking, 2009). Four groups were distinguished from strains with the same morphological characteristics with some representatives of each group chosen for molecular and extrolite analyses. Other isolates belonging to other genera were identified according to Pitt and Hocking (2009) and Samson, Houbraken, Thrane, Frisvad, and Andersen (2010).

2.5. Molecular analysis

A total of 42 *Aspergillus* section *Flavi* strains isolated from samples of different stages of the production chain were subjected to sequencing of a portion of the β-tubulin gene. The methodologies used for DNA extraction, PCR amplification and sequencing were the same as described by Gonçalves et al. (2012). To confirm the taxonomy, the sequences here obtained were aligned with those from type or neotype strains of all recognized species in *A.* section *Flavi*, taking into consideration the novelties of the species accepted in *A.* section *Flavi* provided by Samson et al. (2014), using Clustal W (Thompson, Higgins, & Gibson, 1994). The phylogenetic tree was inferred using the neighbor-joining methods (Saitou & Nei, 1987) and the software package MEGA6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013).

2.6. Extrolite analyses

The extrolite analyses were performed according to Frisvad and Thrane (1987) with Houbraken, Spierenburg, and Frisvad (2012) modifications. Twenty-one representative isolates were grown for 7 and 14 days at 37 °C on both CYA and Yeast Extract Sucrose Agar (YESA). These isolates were chosen according to the growth response as described in item 2.4. Five plugs were taken from each medium and the extrolite was extracted with 0.75 mL of ethyl acetate/dichloromethane/methanol (3:2:1) (v/v/v) with 1% (v/v) of formic acid using 50 min ultrasonication. The solvents were evaporated and the dry extract re-dissolved in 0.4 mL methanol. After filtration the extracts were analyzed using high performance liquid chromatography (HPLC) with diode array detection. The extracts were also analyzed by ultra high performance liquid chromatography (UHPLC) with a maXis 3G Q-TOF orthogonal mass spectrometer (Bruker Daltronics, Bremen, Germany) as described by Klitgaard et al. (2014). The retention time of the compounds was compared to authentic standards (Klitgaard et al., 2014; Kildgaard et al., 2014), and also compared to data in Nielsen, Månsson, Rank, Frisvad, & Larsen, 2011, especially the supplementary data).

2.7. Aflatoxin production potential by Aspergillus section Flavi isolates

The aflatoxin production potential was tested according to the methodology described by Filtenborg, Frisvad, and Svendsen (1983). *Aspergillus* section *Flavi* isolates were inoculated onto YES at 25 °C for 7 days. Then, fungal extracts were taken as plugs and placed on thin layer chromatography (TLC) plates with a mixture of aflatoxin B_1, B_2, G_1 and G_2 standard (Sigma Aldrich, St. Louis, MO, USA), developed in a toluene: ethyl acetate: formic acid 90%: chloroform (7:5:2:5, v/v/v/v) mobile phase, and visualized under UV light at 365 nm.

2.8. Aflatoxin analysis in peanut samples

The aflatoxin analysis was carried out according to Stroka, Anklam, Jorissen, and Gilbert (2000) with modifications.

2.8.1. Clean-up

Twenty-five grams of ground peanuts were added to 2.5 g of NaCl and extracted with 100 mL of methanol: water solution (8:2, v/v) for 30 min. at high speed (10,000 rpm) using a horizontal shaker (New Brunswick Scientific Company, USA). The solution was filtered through quantitative filter paper (Nalgon, Germany), followed by another filtration through glass microfiber filter (Vicam, Sweden). Then, 10 mL of the filtrate were diluted in 60 mL phosphate buffered saline (pH 7.0) and applied to an immunoaffinity column (Aflatest WB Vicam, USA) at a flow rate of 2–3 mL/min. The column was then washed with 30 mL of distilled water and aflatoxins eluted with 1250 μL of methanol and 1750 μL of Milli-Q water.

2.8.2. Chromatographic conditions

Shimadzu LC-10VP HPLC system (Shimadzu, Japan) was used with a reverse phase column and fluorescence detection at 362 nm excitation and 455 nm emission. The system was associated with a Kobracell electrochemical reactor (R-Biopharm, Germany) connected to a current of 100 μA for post-column derivatization of aflatoxins B_1 and G_1. The mobile phase was water: acetonitrile: methanol (6:2:3, v/v/v), with 119 mg of KBr and 350 μL of 4 M nitric acid per liter at a flow rate of 1 mL/min.

2.8.3. Methodology validation

Detection and quantification limits were determined according to Eurachem Guides (1998). To determine aflatoxin recovery on peanut samples, tests were carried out with three different levels of contamination being used (0.5, 5.0 and 25 μg/kg) in triplicate.

3. Results

3.1. Water activity values, fungal infection in peanuts and fungal counts in soil samples

Results of the frequency of occurrence (number of samples that contained a fungal species/total of samples evaluated), average infection (sum of infection level/total number of samples), range of infection (range of infected grains in a sample) and the water activity of the samples are given in Table 1. The mean values of a_w of the soil samples analyzed was 0.979 (0.952–0.999). The peanut production steps from field to ready-to-eat resulted in a mean water activity reduction in the samples collected from 0.859 to 0.394. The water activity of peanuts during the field stage ranged from 0.625 to 0.998 (mean 0.859), while the samples collected at uprooting and windrow presented the higher mean values (0.995 and 0.955 respectively). In addition, the samples collected at the transport step had the lowest water activity at this stage (0.745). The drying stage resulted in a_w decrease providing the microbiological stability for peanuts, with an average value of 0.538 (0.471–0.652). During threshing, sorting and blanching the mean a_w values were 0.571, 0.570 and 0.461 respectively, whereas ready-to-eat samples showed a_w of 0.394.

Peanut mycobiota varied depending on the production stage. In the field samples, *A. flavus*, *Cladosporium* sp., dematiaceous fungi and *Mycelia sterilia* were the most common fungi as they were found in >30% of samples. The range of *A. flavus* infection was from 0 to 90%, showing that this species is well adapted in peanuts and that the infection takes place while still in the field. *Fusarium* and *Eurotium* species occurred in 22% of peanut samples collected in the field; however, one sample was infected with 60% of *Fusarium* while no samples showed *Eurotium* infection higher than 6%. *A. flavus*, *A.* section *Nigri*, *Eurotium* sp., and other *Mycelia sterilia* continued to be isolated from most samples collected at drying, threshing, sorting and blanching steps, although in some the range of infection was low. Some peanut ready to eat samples showed *A. flavus* infection but at a low range (0–8%).

The fungal counts in soil samples were low (10^2 CFU/g) as *A. flavus* was present in three samples. From eight isolates of *A. flavus*, none were aflatoxin B producers while a single *Aspergillus parasiticus* isolate produced aflatoxins B and G.

A total of 556 *A.* section *Flavi* was isolated from the peanut production chain and four different species were identified using morphological, physiological, extrolite and molecular data: *Aspergillus caelatus*, *A. flavus*, *A. parasiticus* and *A. tamarii*. Fig. 1 shows the β-tubulin gene sequence relationship of 42 strains isolated from peanut samples with those from type or neotype strains of all recognized species in *A.* section *Flavi* (Samson et al., 2014).

A. flavus was the most common species isolated throughout the production chain and had the highest number of isolates (n = 515) of which 50% were aflatoxin B_1 and B_2 producers (Table 2). Its occurrence was more frequent in the samples at the sorting stage (78.3%), followed by drying (63.2%), threshing (54.5%), blanching (47.6%), field (40.7%) and ready-to-eat (31.3%). The average percentage of infection varied from 12.5% during drying to 1.3% in ready-to-eat samples.

A. parasiticus (n = 17 isolates) was found in samples collected in the field, drying and sorting stages with a frequency of occurrence of 7.9, 21.1 and 20% and average of infection of 0.2, 0.6 and 0.5% respectively. *Aspergillus caelatus* (11 isolates) was present at almost all stages except for ready-to-eat samples. The frequency of occurrence and average of infection was of 7.4, 5.26 and 4% and 0.52, 0.31 and 0.08% for peanut samples collected in the field, drying and sorting, respectively. *Aspergillus tamarii* (13 isolates) was present in peanut samples collected at all stages, except in those collected in field and threshing, and most frequent at sorting (12%) with an average of infection of 0.5%.

3.2. Extrolite analyses

The findings of the extrolite analyses are given in Table 2. Among the seven *A. flavus* strains tested, six (86%) were cyclopiazonic acid producers, five (71%) produced kojic acid, four (57%) produced ditryptophenaline, three (43%) produced flavimin and miyakamides and one (14%) produced 3-*O*-methylsterigmatocystin, an aflatoxin precursor. Ten *A. parasiticus* strains were tested and nine (90%) were aspergillic acid producers and eight (80%) produced kojic acid. Among the extrolites, ditryptophenalins, miyakamide, parasiticolides and 3-*O*-methylsterigmatocystin were also found. Tenuazonic acid was produced by one strain of *A. parasiticus*. Two strains of *A. caelatus* were

Table 1
Frequency of occurrence, mean and variation in the level of infection by fungi at different stages of the peanut chain.

Stage of peanut chain (number of samples)	Field (27)			Drying (19)			Threshing (11)			Sorting (25)			Blanching (21)			Ready to eat (16)		
Mean a_w (range)	0.859 (0.625–0.998)			0.538 (0.471–0.652)			0.570 (0.482–0.664)			0.570 (0.457–0.690)			0.461 (0.404–0. 639)			0.394 (0.287–0.652)		
Fungi	FO (%)	AI (%)	RI (%)	FO (%)	AI (%)	RI (%)	FO (%)	AI (%)	RI (%)	FO (%)	AI (%)	RI (%)	FO (%)	AI (%)	RI (%)	FO (%)	AI (%)	RI (%)
Aspergillus caelatus	7.4	0.52	0–8	5.26	0.31	0–6	0	0	0	4	0.08	0–2	0	0	0	0	0	0
Aspergillus flavus	37	7.11	0–90	68.42	12.63	0–46	63.64	5.81	0–20	80	14.24	0–56	47.62	7.52	0–44	31.25	1.25	0–8
Aspergillus parasiticus	11.11	0.37	0–6	21.05	0.73	0–6	0	0	0	16	0.4	0–4	0	0	0	0	0	0
Aspergillus tamarii	0	0	0	10.52	0.21	0–2	0	0	0	8.7	0.26	0–4	4.76	0.28	0–6	6.25	0.12	0–2
A. terreus	3.7	0.15	0–4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aspergillus section *Nigri*	25.93	2.29	0–24	84.21	6.1	0–36	63.64	3.81	0–10	80	7.92	0–24	33.3	1.05	0–6	12.5	0.37	0–4
Penicillium sp.	0	0	0	5.26	0.21	0–4	0	0	0	12	0.32	0–4	4.76	0.09	0–2	0	0	0
Fusarium sp.	22.22	3.26	0–60	10.53	0.74	0–12	9.1	1.09	0–12	4	0.24	0–6	0	0	0	0	0	0
Eurotium sp.	22.22	0.59	0–6	42.1	1.68	0–30	72.72	26.9	0–82	44	14.8	0–82	33.3	2.57	0–26	25	3	0–26
Another ascomycetes	3.70	0.07	0–2	5.26	0.5	0–10	0	0	0	0	0	0	0	0	0	0	0	0
Cladosporium sp.	44.44	2.15	0–16	26.31	1.16	0–8	27.28	0.54	0–2	4	0.08	0–2	4.76	0.09	0–2	6.25	0.25	0–4
Syncephalastrum sp.	0	0	0	0	0	0	0	0	0	4	0.08	0–2	0	0	0	0	0	0
Rhizopus sp.	14.81	1.85	0–30	31.58	1.68	0–12	18.18	1.27	0–8	16	1.76	0–18	14.29	1.14	0–20	0	0	0
Mucor sp.	3.7	0.07	0–2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Neurospora sp.	0	0	0	5.26	0.53	0–10	0	0	0	0	0	N	0	0	0	0	0	0
Dematiaceos fungi	40.74	6.59	0–32	21.05	1.37	0–18	36.36	8.54	0–44	44	3.44	0–20	19.05	1.05	0–14	0	0	0
Mycelia sterilia	48.15	10.37	0–48	73.68	10.21	0–40	63.63	9.1	0–24	80	10.4	0–34	57.14	3.14	0–12	18.75	1.75	0–14
Wallemia sp.	0	0	0	0	0	0	9.1	0.72	0–8	0	0	0	19.05	2.38	0–28	18.75	5.37	0–36

FO = frequency of occurrence % (number of samples that contained a fungi species / total of samples evaluated); AI = average of infection (sum of infection level/total number of samples); RI = range of infection % (range of infected grains in a sample).

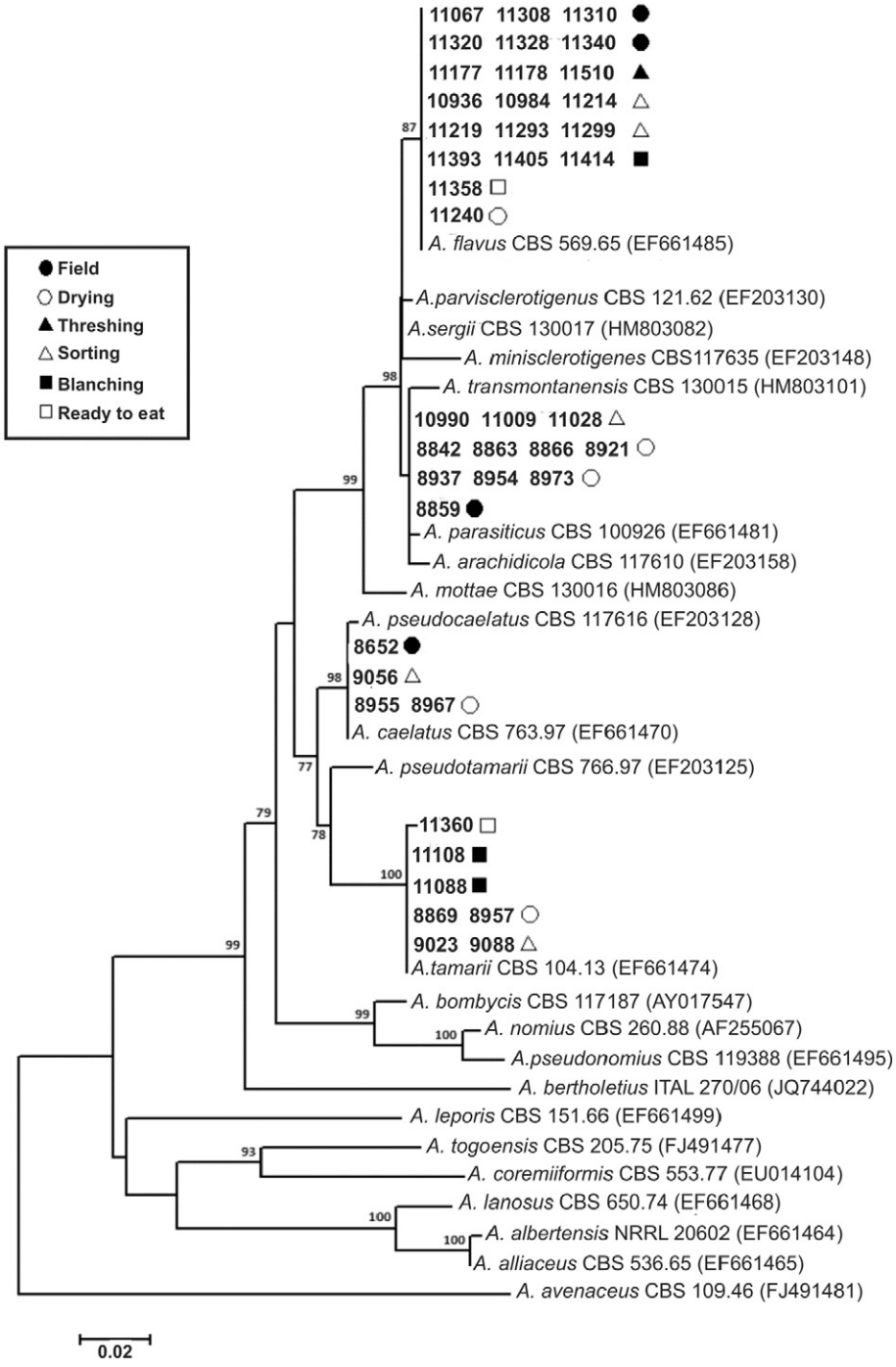

Fig. 1. Neighbor-Joining tree based on β-tubulin sequence data of *Aspergillus* section *Flavi* type strains and *Aspergillus* isolates from different stages of the peanut chain (identified by geometric symbols). Nodes supported by bootstrap values > 70% are indicated by numeric values.

tested and both produced aspergillic acid (weak), aspirochlorin, kojic acid, and SCYT.

3.3. Aflatoxin analyses

Table 3 shows the results of the aflatoxin analyses performed in the samples collected throughout the peanut chain. The detection and quantification limit for aflatoxin B_1, B_2, G_1 and G_2 were 0.02, 0.01, 0.01 and 0.01 μg/kg and 0.06, 0.05, 0.04 and 0.02 μg/kg respectively. The recovery percentage of samples contaminated with 0.5, 5.0, and 25 μg/kg was: 96.8, 88.2 and 99.4% respectively. The mean values of contamination were presented as lower bound (Lb), where the values below the limit of detection (LOD) were replaced by zero and upper bound (Ub) where the values were replaced by LOD divided by 2. Aflatoxin was present in almost 10% of the samples analyzed, except in the samples collected at the field and threshing stages.

The average of contamination of peanut samples collected at drying, blanching, sorting and ready to eat were 7.63 μg/kg, 0.3 μg/kg, 5.06 μg/kg and 1.29 μg/kg, respectively. Separated values from each positive sample and aflatoxigenic fungal incidence are detailed in Table 4. The highest concentration of total aflatoxin was found in samples at sorting stage with 100.91 μg/kg, followed by those collected at drying stage, that presented 95.46 μg/kg of this mycotoxin. The concentration of aflatoxins in ready-to-eat samples ranged from 14.07 μg/kg to 0.82 μg/kg.

Table 2
Percentage of *Aspergillus* section *Flavi* aflatoxin producer isolates and other polyphasic characteristics.

	No isolates	% Aflatoxin producers (type of aflatoxins produced)	Extrolites	Reverse AFPA
A. caelatus	11	0	Aspergillic acid, aspirochlorin, kojic acid, tenuazonic acid	Yellow green
A. flavus	515	50 (B_1 and B_2)	Cyclopiazonic acid, flavimin[a], miyakamides, kojic acid, 3-*O*-methylsterigmatocystin	Bright orange
A. parasiticus	17	100 (B_1, B_2, G_1 and G_2)	Aspergillic acid, ditryptophenalins, kojic acid, miyakamide, parasiticolides, 3-*O*-methylsterigmatocystin	Orange
A. tamarii	13	0	Not analyzed	Red brown

[a] A diketopiperazine that is unique to *Aspergillus flavus*.

4. Discussion

In our study the wide variety of fungi isolated in peanut samples during the production chain can be explained by variations in water activity and related production stages. Samples from the field had a higher water activity average when compared to other steps, which favored mold growth mainly of the genera *Mycelia sterilia*, *Aspergillus* species, dematiaceous fungi, *Cladosporium*, *Eurotium* and *Fusarium*. A similar mycobiota was reported by Gonçalez et al. (2008) that analyzed peanut samples from different maturity stages and after drying with the most commonly observed being *Fusarium* spp. (26%), *A. flavus* (17%), *A. terreus* (9.5%) and *Penicillium* spp. (5%).

The results observed in the soil samples were similar to Horn and Dorner (1998) that studied the soil population of *Aspergillus* section *Flavi* in peanut fields and found that *Aspergillus flavus* and *Aspergillus parasiticus* were the dominant species in the soil.

Four species of *Aspergillus* section *Flavi* were identified from samples at different production stages: *A. caelatus*, *A. flavus*, *A. parasiticus* and *A. tamarii*. These species have been reported in other peanut mycobiota studies (Pitt et al., 1993; Pitt et al., 1998; Nakai et al., 2008; Gonçalez et al., 2008). Although Pildain et al. (2008) showed the occurrence of *A. arachidicola* and *A. minisclerotigenes*, which were described as aflatoxin-producing species in *A.* section *Flavi*, these species were not found in this study. Fungal extrolites and/or molecular technique were helpful for confirming the identification of these isolates. All *Aspergillus* species have unique DNA characters and produce a unique combination of different types of extrolites; some of these compounds are even unique to a single species (Frisvad, 1989; Samson & Varga, 2009).

The average of *A. flavus* aflatoxin B producer isolates was 50% and ranged from 31% at the threshing stage to 83% in the field. These results agree with Frisvad, Thrane, and Samson (2007) and Taniwaki and Pitt (2013) that reported about 40% of *A. flavus* isolates were aflatoxin B producers. Other studies showed higher percentages of *Aspergillus flavus* aflatoxin producers. For instance, Vaamonde et al. (2003) studied strains isolated from different substrates including peanuts (37 strains) and observed that the incidence of aflatoxigenic *A. flavus* strains was 73% in

Table 3
Concentration (μg/kg) of aflatoxins B_1, B_2, G_1 and G_2 in peanut samples at different stages of chain production.[a]

Stage of peanut chain	Aflatoxin B_1	Aflatoxin B_2	Aflatoxin G_1	Aflatoxin G_2	Total aflatoxins
Field (27)					
Mean (Lb)	<LOD	<LOD	<LOD	<LOD	<LOD
Mean (Ub)	0.01	0.005	0.005	0.005	0.025
Median	<LOD	<LOD	<LOD	<LOD	<LOD
Range	<LOD	<LOD	<LOD	<LOD	<LOD
No of positive samples	0	0	0	0	0
Drying (19)					
Mean (Lb)	6.534	1.102	0.004	<LOD	7.64
Mean (Ub)	6.54	1.11	0.009	0.006	7.66
Median	<LOD	<LOD	<LOD	<LOD	<LOD
Range	<LOD–83.26	<LOD–12.21	<LOD–0.07	<LOD	<LOD–95.48
No of positive samples	3 (16%)	2 (11%)	1 (5%)	0	3 (16%)
Threshing (11)					
Mean (Lb)	<LOD	<LOD	<LOD	<LOD	<LOD
Mean (Ub)	0.01	0.005	0.005	0.005	0.025
Median	<LOD	<LOD	<LOD	<LOD	<LOD
Range	<LOD	<LOD	<LOD	<LOD	<LOD
No of positive samples	0	0	0	0	0
Sorting (25)					
Mean (Lb)	3.946	0.201	0.897	0.016	5.06
Mean (Ub)	3.95	0.21	0.90	0.022	5.08
Median	<LOD	<LOD	<LOD	<LOD	<LOD
Range	<LOD–76.71	<LOD–1.36	<LOD–22.43	<LOD–0.41	<LOD–100.92
No of positive samples	4 (16%)	2 (8%)	1 (4%)	1 (4%)	4 (16%)
Blanching (21)					
Mean (Lb)	0.15	0.06	0.07	0.02	0.30
Mean (Ub)	0.16	0.06	0.08	0.02	0.32
Median	<LOD	<LOD	<LOD	<LOD	<LOD
Range	<LOD–3.13	<LOD–1.24	<LOD–1.56	<LOD–0.41	<LOD–6.34
No of positive samples	1 (5%)	1 (5%)	1 (5%)	1 (5%)	1 (5%)
Ready to eat (16)					
Mean (Lb)	1.13	0.157	<LOD	<LOD	1.29
Mean (Ub)	1.14	0.16	0.01	0.01	1.31
Median	<LOD	<LOD	<LOD	<LOD	<LOD
Range	<LOD–12,77	<LOD–1,28	<LOD	<LOD	<LOD–14,05
No of positive samples	4 (25%)	4 (25%)	0	0	4 (25%)
Limit of detection (LOD)	0.02	0.01	0.01	0.01	0.05
Limit of quantification (LOQ)	0.06	0.05	0.04	0.02	0.17

[a] Lb: lower bound; Ub: upper bound.

Table 4
Peanut samples with highest aflatoxin (μg/kg) and aflatoxigenic fungi incidence at each stage of the production chain.

Stage of peanut production Sample number	Aflatoxins (μg/kg)					a_W	*A.* section *Flavi* infection (%)
	B_1	B_2	G_1	G_2	Total		
Drying							
Sample # 75	83.26	12.2	N.D.	N.D.	95.46	0.569	8% *A. flavus*
Sample # 76	0.35	N.D.	0.07	N.D.	0.43	0.602	38% *A. flavus*, 4% *A. parasiticus*
Sample # 2975	40.53	8.73	N.D.	N.D.	49.26	0.471	46% *A. flavus*
Blanching							
Sample # 5597	3.13	1.24	1.56	0.41	6.34	0.444	0% *A.* section *Flavi*
Sorting							
Sample # 90	0.82	N.D.	N.D.	N.D.	0.82	0.585	56% *A. flavus*, 2% *A. parasiticus*
Sample # 77	76.71	1.36	22.43	0.41	100.91	0.690	4% *A. flavus*
Sample # 78	0.3	N.D.	N.D.	N.D.	0.3	0.682	4% *A. flavus*
Sample # 2944	20.80	3.66	N.D.	N.D.	24.46	0.605	14% *A. flavus*
Ready to eat							
Sample # 5602	12.77	1.28	N.D.	N.D.	14.075	0.342	0% *A.* section *Flavi*
Sample # 5604	0.68	0.120	N.D.	N.D.	0.82	0.341	0% *A.* section *Flavi*
Sample # 5605	3.38	0.801	N.D.	N.D.	4.21	0.376	0% *A.* section *Flavi*
Sample # 5606	1.29	0.304	N.D.	N.D.	1.62	0.359	0% *A.* section *Flavi*

peanuts, and a study conducted by Schroeder and Boller (1973) showed that from 419 strains of *A. flavus*, 96% were aflatoxin B producers. The presence of *A. flavus*, which is potentially an aflatoxin producer throughout all the production chain, including ready-to-eat samples, highlight the importance of good storage with temperature and moisture control in order to avoid ideal conditions for aflatoxin production.

Aflatoxins were found in this study in samples throughout the peanut production chain. From eight positive samples collected at the drying, blanching and sorting stages, four samples were above the limit established by European and Brazilian regulations (24.46, 49.26, 95.46 and 100.91 μg/kg). Two samples at the ready-to-eat stage showed amounts of total aflatoxins above the European regulation (14.07 and 4.21 μg/kg), but according to the Brazilian regulation the values were acceptable which consequently could lead to higher exposure of the Brazilian population to this toxin. The peanut sample with the highest total aflatoxin contamination (100.91 μg/kg) belonging to the sorting stage was collected during the selection by density and belonged to the smaller grains. In Brazil, during this selection stage, smaller grains are sent for the production of oil. During the refining process for obtaining oil, the mycotoxin is removed so, despite having high contamination by aflatoxins, it would not present a direct risk to the consumer (Parker & Melnick, 1966).

Finally, the presence of aflatoxigenic strains and aflatoxins in peanut samples did not always show a relationship between them. The reduced a_W indicated that the sample had already been dried, reducing the levels of viable fungi. Fungi with potential to produce aflatoxin rarely grow below 0.80. However, the toxin remains in the peanut.

5. Conclusion

The data reported in this study add information on the occurrence and biodiversity of fungi in peanuts, especially those belonging to *A.* section *Flavi*, at several stages of the production chain. Furthermore, the occurrence of aflatoxins is also of major relevance for continuous monitoring and assessment of likely exposure of consumers to this mycotoxin through consumption of peanuts.

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